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(54) Title: METHOD FOR THE TREATMENT OF NEUROLOGICAL AND NEUROPSYCHOLOGICAL DISORDERS

(57) Abstract: The present invention discloses a method for therapeutically treating an animal, including a human, for psychosomatic, depressive and neuropsychiatric diseases, such as anxiety, depression, insomnia, schizophrenia, epilepsy, spasm and chronic pain. Administration of a suitable attractin inhibitor causes the reduction of activity in the enzyme attraction or in isoforms thereof in the brain of mammals and leads as a causal consequence to a reduced degradation of the neuropeptide Y (NPY) and similar substrates. Such treatment will result in a reduction or delay in the decrease of the concentration of functionally active neuronal NPY (1-36). As a consequence of the resulting enhanced stability of the endogenous NPY(1-36), NPY activity is prolonged thereby resulting among other things in functionally active NPY Y1 receptor activity thereby facilitating antidepressive, anxiolytic, analgesic, antihypertension and other neurological effects.

Method for the treatment of neurological and neuropsychological disorders.

BACKGROUND OF THE INVENTION

Field of the invention

The present invention relates to the function of attractin and of attractin isoforms within the central nervous system (CNS) and their biological effects on neuropeptide levels, neurotransmission and behavior. The present invention also relates to the potentiation of endogenous neurological and neuropsychological effects of brain neuropeptide Y (NPY) systems and other substrates of attractin by selective inhibition of attractin and of attractin isoforms. The invention relates further to the treatment of hypertension, fever, sleep dysregulation, anorexia, anxiety related disorders including depression, seizures including epilepsy, drug withdrawal and alcoholism, neurodegenerative disorders including cognitive dysfunction and dementia, and neuropsychiatric disorders including schizophrenia, via a potentiation of NPY Y1 receptor mediated effects resulting from an inhibition of attractin and of attractin isoforms within the CNS.

Background Art

Discovery of NPY

Neuropeptide Y (NPY), a 36 amino acid peptide belonging to the pancreatic polypeptide family, was first isolated from porcine brain in 1982 (Tatemoto and Mutt, 1982). NPY is present in all sympathetic nerves innervating the cardiovascular system and is the most abundant peptide in the brain and the heart. Additionally, in rats, but not in humans, NPY is also found extraneuronally in platelets and endothelium (Zukovska-Grojec et al., 1993). Originally, NPY was known as a potent vasoconstrictor and a neuromodulator. Released by stress, exercise, and myocardial ischemia, NPY has been implicated in coronary heart disease, congestive heart failure, and hypertension (Zukovska-Grojec et al, 1998). More recently, because of the potent ability of NPY to stimulate food intake, it is suspected to play a role in obesity and diabetes (Kalra et al., 1999). Latest findings indicate that NPY is also a mitogen for rat aortic vascular smooth muscle cells (Zukovska-Grojec et al., 1999).

NPY-related research has focussed on at least three main directions: (1) Co-transmission and sympathetic vasoconstriction, because of its co-expression with noradrenaline; (2) neurotransmission and function within the CNS, because of potent consummatory effects; and (3) evolution of NPY, since NPY is one of the most highly conserved bio-active peptides known (Colmers and Wahlestedt, 1993;

Lundberg, 1996; Wahlestedt and Reis, 1993; Wettstein et al., 1996). NPY acts on at least six receptors (Y1-Y6), with varying peptide pharmacology and distinct distribution in the CNS (Gehlert, 1998) (Tab. 1).

Distribution of NPY, NPY receptor subtypes and mRNA

The distribution of NPY itself, NPY receptor protein and their mRNA within the CNS of human and rat brains has recently been reviewed (Dumont Y, Jacques D, St-Pierre, J.-A., Tong, Y., Parker, R., Herzog H. and Qurion, R., 2000; in Handbook of Chemical Neuroanatomy, Vol. 16: Peptide Receptors, Part I; Quirion, R., Björklund, A. and Hökfeld, T., editors). A brief survey is given in Tab. 1.

NPY-containing neurons are evident in the nasal mucosa of various species including man, often associated with glandular acini and blood vessels (Baraniuk et. Al., 1990; Grunditz et. al., 1994). Stimulation of the parasympathetic nerve supply to the nasal mucosa (vidian nerve) in dogs increases blood flow in the region and causes mainly atropine resistance. Intravenous administration of NPY reduces vasodilitation due to parasympathetic nerve stimulation, an effect that was not mimicked by the NPY Yl- selective agonist [Leu31, Pro34]NPY, but was mimicked by administration of the NPY Y2- receptor agonist N-acetyl[Leu28,Leu31JNPY(24-36) (Lacroix et al., 1994). This is consistent with a prejunctional NPY Y2- like receptor-mediated inhibition of transmitter release from parasympathetic nerve terminals.

NPY receptor function

NPY is unarguably the most abundant neuropeptide discovered to date, with a wide distribution in the CNS and the peripheral nervous system (PNS). NPY forms a family of peptides together with peptide YY (PYY) (approximately 70% homology) and pancreatic polypeptide (PP) (approximately 50% homology); both NPY and PYY are extremely bio-active, whereas PP is generally much less active (Gehlert, 1998; Wahlestedt and Reis, 1993) (Tab. 2).

Two receptor subtypes of NPY have been called neuropeptide Y Yl (postjunctional) and neuropeptide Y Y2 (prejunctional) on the basis of the different responses to a truncated analog of the related peptide YY- (13- 36), when compared with neuropeptide Y in *in vitro* assay systems (Wahlestedt et al., 1986). Activation of neuronal prejunctional NPY receptors generally inhibits nerve activity, reducing the release of neurotransmitters in response to nerve impulses and in response to local factors acting to release neurotransmitters (Wahlestedt et al., 1986). The prejunctional or neuropeptide Y Y2 receptor classification was based on actions of peptide YY (13-36) but in many systems this molecule, as well as neuropeptide Y-(13-36), does exhibit pressor activity (Rioux et al., 1986; Lundberg, et al., 1988; Potter et al., 1989). This has been interpreted by some to indicate that in some vascular beds there are two types of neuropeptide Y receptors (both neuropeptide Y Yj and neuropeptide Y2) on postjunctional

membranes (Schwartz et al., 1989). However the lack of selectivity of these molecules may be due to retention of partial agonistic activity on Yj receptors, which permits them to evoke a reduced functional response. Previously, a 13-36 analog of neuropeptide Y, (Leu 17 , Glu", Ala 21 , Ala 22 , Glu 23 , LeU28, LeU31) neuropeptide Y- (13-36) (ANA neuropeptide Y-(13-36)) which displayed prejunctional activity equivalent to the whole neuropeptide Y molecule in studies *in vivo* was described (Potter et al., 1989).

Apart from these historically well-defined neuropeptide Y receptors the existence of a number of other subtypes (Y3, Y4, Y5 and Y6) has been suggested on a pharmacological basis (Michel et al., 1998) and details of the cloning of receptors corresponding to Y1, Y2, Y4 and Y5 have been published (Herzog et al., 1992; Gerald et al., 1995; Bard et al., 1995; Gerald et al., 1996) (Tab. 1). The distribution and physiological significance of these various receptor subtypes has yet to be defined. Although some controversy has existed about the selectivity of truncated forms of neuropeptide Y for one or other receptor subtype (Potter et al., 1989), the emerging picture supports the initial classification into pre- and postjunctional receptor subtypes. Cell lines have been developed which express specifically one neuropeptide Y receptor subtype and the development of receptor- selective analogs of neuropeptide Y has focussed mainly on binding characteristics in these cell lines (Sheikh et al., 1989; Aakerlund et al., 1990; Fuhlendorff et al., 1990). More recently, a cDNA encoding the neuropeptide Y Y1 receptor has been cloned and cell lines expressing the cloned receptor have been analyzed for both specific binding of neuropeptide Y analogs (Herzog et al., 1992) and functional responses elicited by specific analogs. From such binding studies, combined with subsequent studies *in vivo*, two analogs have been classified as acting specifically on the postjunctional neuropeptide Y Y1 receptor. These neuropeptide Y Y receptor selective analogs, (Pro 34) neuropeptide Y and (Leu", Pro 34) neuropeptide Y, mimic the action of neuropeptide Y in raising blood pressure, and also share similar binding to cell lines expressing only neuropeptide Y Y receptors e.g. the human neuroblastoma cell line SK-N-MC and fibroblast lines expressing the cloned neuropeptide Y Y, receptor (Herzog et al., 1992). Neither exhibits the neuropeptide Y Y2 receptor action an inhibition of cardiac vagal action *in vivo*, a manifestation of inhibition of acetylcholine release (Potter et al., 1991; Potter and McCloskey, 1992).

Table 1: DISTRIBUTION AND FUNCTION OF NPY RECEPTOR SUBTYPES WITHIN THE CNS

Receptor-subtype	CNS Expression	Function	Selective Agonist	Selective Antagonist or selectivity
Y1	Cortex, etc.	Anxiolysis, LHRH Release	Intact N - Terminus: [Leu31,Pro34]NPY	BIBP3226; BIBO 3304
Y2	Hippocampus, Hypothalamus	Antiamnestic	C-terminale End: PYY3-36; PYY13-36	T4[NPY(33-36)]4; BIIE0246
Y3	Ncl. Tractus Solitarius (NTS)	Bradycardia, Hypotension	NPY>>PYY, [Leu31,Pro34]NPY	PYY - Insensitivity
Y4	Dorsal vagal Complex (DVC)	Emetic	PP>>NPY, PYY	PP – Preferring
Y5 (a)	Hypothalamus	Feeding	NPY, PYY, [Leu31,Pro34]NPY	[Leu31,Pro34]NPY - sensitive, BIBP3226 - non-reversible
Y5 (b) or Y6	Hypothalamus	?; species specific	?	?

Tab. 1: NPY Receptor subtypes within the CNS; ? = unknown or not investigated

The development of the high affinity, non-peptide NPY antagonists, BIBP3226 and BIBO3304, has facilitated the functional characterization of NPY receptors, as this compound shows selectivity for Y1R, being devoid of activity on at least Y2R, Y3R and Y4R (Doods et al., 1996). Recently, a two Y2 receptor antagonist has been described. One is a TASP-molecule (Grouzmann et al., 1997), the other a non-peptide antagonist (Wieland et al., 1999) and other non-peptide receptor specific compounds became available (Daniels et al., 1995). Thus, specific receptor blockade within the brain would allow the functional characterization of behavioral and physiological effects mediated by central NPY receptors. In addition, mice lacking the Y1R were generated and are available (Pedrazzini et al., 1998). Neurons showing NPY-like immunoreactivity and NPY receptor expression are abundant in the CNS (Tab. 1), and perhaps are most notably found in hypothalamic and so-called limbic structures, but are also co-localized with brain stem monoaminergic neurons and cortical GABA-ergic neurons (Chronwall, 1985; Dumont et al., 1996).

TABLE 2: RECEPTOR SUBTYPES AND PEPTIDE SELECTIVITY	
Receptor subtype	Peptide Potency
Y1-like	
Y1	NPY = PYY = Pro^{34}-NPY > PP > NPY_{13-36}
Y4	PP >> NPY = PYY = LP-NPY > NPY_{13-36}
Y6	NPY = PYY = Pro^{34}-NPY > NPY_{13-36} > PP
Y2-like	
Y2	NPY = PYY = NPY_{13-36} > Pro^{34}-NPY > PP
Y5-like	
Y5	NPY = PYY = Pro^{34}-NPY > NPY_{13-36} > PP
Not cloned	
PP receptor	PP >> PYY = NPY
Y3	NPY = Pro^{34}-NPY = NPY_{13-36} >> PYY
PYY-preferring	PYY > NPY >> NPY_{13-36} >> Pro^{34}-NPY

Tab. 2: Receptor subtypes and peptide selectivity according to Gehlert, 1998.

NPY, anxiety and depression

Anxiolytic-like effects of NPY have been demonstrated using the elevated plus maze test (Montgomery), the punished drinking test (Vogel), and the punished responding test (Geller-Seifter), with potency and efficacy matching those of benzodiazepines (Griebel, 1999; Heilig et al., 1989; Wettstein et al., 1995). NPY acts anxiolytic-like on the response to novelty (Heilig and Murison, 1987; von Hörsten et al., 1998b), and produces anxiolytic-like effects on the elevated plus maze and other anxiety related tests (Wahlstedt and Reis, 1993; Wahlestedt et al., 1993). Interestingly, Y1 receptor antisense-treated rats showed marked anxiety-related behaviors, without alterations of locomotor activity and food intake (Wahlestedt et al., 1993). Additionally, in the Flinder rat strain, a genetic model of depression, Y1 receptor mRNA expression was decreased in different cortical regions and the dentate gyrus of the hippocampus, while Y2 receptor mRNA expression did not differ from controls (Caberlotto et al., 1998). Olfactory bulbectomy in the rat has been developed as a model of depression (Leonard and Tuite, 1981). In this model, most of the changes resemble those found in depressed patients (Song et al., 1996). A 7-day i.c.v. administration of NPY in olfactory bulbectomized rats attenuated behavioral and neurotransmitters deficits in this model (Song et al., 1996). NPY Y1, Y2, and possibly Y5 receptors, seem to be involved in the regulation of anxiety levels in rodents, with Y1-mediated effects being best characterized (Heilig et al., 1993; Kask et al., 1998b). It can be concluded, therefore, that endogenous NPY counteracts stress and anxiety (Heilig et al., 1994). Furthermore, these data suggest that the Y1 receptor subtype could be implicated in anxiety- and depression-related behaviors. Additionally, Kask et al. (1996) reported that i.c.v. injection of the Y1 antagonist, BIBP3226, produced anxiogenic-like effects in the elevated plus-maze test, without any locomotor deficit. This effect can be reproduced by the administration of BIBP3226 in the dorsal periaqueductal gray matter but not in the locus coeruleus or

the paraventricular nucleus of the hypothalamus (Kask et al., 1998c). Moreover, BIBP3226 and GR231118 administered into the dorsal periaqueductal gray matter decreased the time spent in active social interaction in rats (Kask et al., 1998d). The brain regions which are important for the anti-stress action of NPY include but may not be limited to the amygdala (Sajdyk et al., 1999, Thorsell et al., 1999), locus coeruleus (Kask et al., 1998c) and dorsal periaqueductal gray (Kask et al., 1998a,b). Amygdala NPY is not released under low stress conditions since blockade of NPY Y_1R with BIBP3226 or BIBO3304 did not increase anxiety as measured in the elevated plus-maze and social interaction tests (Kask et al., 1998b; Sajdyk, 1999). Constant NPY-ergic tone, however, seems to exist in the dorsal periaqueductal gray matter, where the NPY Y_1R antagonist had anxiogenic like effects in both experimental anxiety models (Kask et al., 1998a,b). Thus, in certain brain regions, there may be a tonic regulation of anxiety via NPY systems.

Neurological and psychophysiological effects of CNS NPY systems: Pleiotropy

Thus, numerous studies have addressed the physiological functions of NPY and its congeners in the CNS (for reviews see: Kalra and Crowley, 1992; Dumont et al., 1992; Stanley, 1993; Wahlestedt and Reis, 1993; Grundemar et al, 1993; Gehlert, 1994, 1998; Colmers and Bleakman, 1994; Wettstein et al, 1995; Heilig and Widerlow, 1995; Munglani et al., 1996; Inui, 1999; Bischoff and Michel, 1999; Vezzani et al., 1999) and demonstrated a broad range of effects. No pharmacological approaches exist, at present, to gain advantage of these various physiological functions.

Current problems in the treatment of anxiety related disorders using benzodiazepines or NPY

The current methods for treatment of anxiety are accompanied by several problems:

The benzodiazepines that are commonly used as anxiolytic agents are unnatural compounds with a low or no selectivity. Beside their anxiolytic activity, the benzodiazepines show sedative and anti-epileptic effects and are suspected to influence muscle relaxation. Unfortunately, they are associated with a number of unwanted side effects, namely tiredness, sleepiness, lack of concentration, reduction of attentiveness and reactivity. Chronic application of benzodiazepines causes neurological disorders, like ataxia, dizziness, reflex loss, muscle and language disorders. A long-term treatment with benzodiazepines is predicted to entail dependency and addiction.

The direct i.c.v. administration of neuropeptide Y for the long-term treatment of anxiety in patients is not feasible.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a medicament beneficial for neurological and psychophysiological effects. It is especially an object of the present invention, to provide an inhibitor of attractin or of an attractin isoform for the production of a medicament for modulating behavioral and/or neurological adaptive responsiveness to stress including anxiety.

In addition, it is an object of the present invention to overcome or reduce the above stated problems of the prior art by providing a pharmacological approach and results in a maintained or prolonged activity and/or effect of NPY in the brain of mammals.

These objects are solved by the use of an inhibitor of attractin or of attractin isoforms for the production of a medicament for modulating behavioral and/or neurological responsiveness to stress including anxiety.

This results in the magnification of endogenous neurological or neuropsychological effects mediated by NPY Y1 receptors, including but not limited to a reduction of anxiety, treatment of hypertension, fever, sleep dysregulation, anorexia, anxiety related disorders including depression, seizures including epilepsy, drug withdrawal and alcoholism, neurodegenerative disorders including cognitive dysfunction and dementia, and neuropsychiatric disorders including schizophrenia diagnosed in a subject.

Figure 1 shows MALDI-TOF mass spectra of the proteolytic processing of RANTES 1-15 by DP IV and attractin (A) and NPY by attractin in absence (left side) and presence (right side) of isoleucyl-thiazolidine hemifumarate (P32/98).

DETAILED DESCRIPTION OF THE INVENTION

In contrast to other proposed methods in the art, the present invention provides an orally available therapy with low molecular weight inhibitors of attractin or attractin isoforms (isoenzymes). The instant invention represents a novel approach for the treatment of anxiety and other neurological or psychological disorders in mammals. It is user friendly, commercially useful and suitable for use in a therapeutic regime, especially concerning human disease.

Examples for orally available low molecular weight inhibitors of the attractin enzyme activity are agents such as, N-(N'-substituted glycyl)–2-cyanopyrrolidines, L-*threo*-isoleucyl thiazolidine, L-*allo*-isoleucyl thiazolidine, L-*threo*-isoleucyl pyrrolidine, L-*allo*-isoleucyl thiazolidine, and L-*allo*-isoleucyl pyrrolidine. They are described in US 6, 001, 155, WO 99/61431, WO 99/67278, WO 99/67279, DE 198 34 591, WO 97/40832, DE 196 16 486 C 2, WO 98/19998, WO 00/07617, WO

99/38501, and WO 99/46272, the teachings of which are herein incorporated by reference in their entirety.

Attractin is an enzyme that is an exopeptidase, which selectively cleaves peptides after penultimate N-terminal proline and alanine residues. Presently 5 isoforms of attactin are known. Attractin-like enzymes, which can also be used according to the present inevntion, can, e.g., be selected by subjecting peptidases to a test for selectivity cleaving peptides after penultimate N-terminal proline and alanine residues, selecting a peptidase which effects such a cleavage and isolating the peptidase.

Various effects of attractin inhibitors imply their impact on normal healthy tissues and organs, when they are used for the treatment of a pathologically altered tissue. The goal of the present invention is the development of highly selective brain targeted inhibitors for attractin and of attractin isoforms, which display a high bioavailability and an exactly predictable activity time in the target tissue.

Examples for orally available low molecular weight agents are prodrugs of stable and unstable inhibitors of the attractin enzyme activity which comprise the general formula A-B-C, whereby A represents an amino acid, B represents the chemical bond between A and C or an amino acid, and C represents an unstable or a stable inhibitor of the attractin enyzme activity, respectively. They are described in WO 99/67278, WO 99/67279 the teachings of which are herein incorporated by reference in their entirety.

The present invention relates to a novel method in which the reduction of activity of the enzyme attractin or of attractin isoforms in the brain of mammals induced by effectors of the enzyme leads as a causal consequence to a reduced degradation of the neuropeptide Y (NPY). Such treatment will result in a reduction or delay in the decrease of the concentration of functional active NPY (1-36).

According to the present invention it has been found that the effect and/or activity of NPY in the brain of mammals, especially humans, can be maintained or prolonged by the administration of inhibitors of attractin or of attractin isoforms (isoenzymes). Thereby, the degradation of NPY in the brain can be reduced. This results in an alleviation or improvement of psycosomatic, depressive and/or neuropsychiatric diseases. The instant invention especially represents a novel approach for the treatment of anxiety and other neurological or psychological disorders. It is user friendly, commercially useful and suitable for use in a therapeutic regime, especially concerning human disease.

Surprisingly, the inventors have found that the administration of the attractin inhibitor isoleucyl thiazolidine exhibits an anxiolytic effect.

Attractin and its isoforms are present and widely distributed in rat brain (Lu et al., 1999). The inventor shows in example 1, that NPY is a principal substrate for attractin *in vitro*.

As a consequence of the resulting enhanced stability of the endogenous NPY (1-36) caused by the inhibition of attractin activity, NPY activity is prolonged resulting in functionally active NPY Y1 receptor activity facilitating – among others – anti-depressive, anxiolytic and anti-hypertensive effects (see above).

The method of the present invention for treating anxiety in an animal, including humans, in need thereof, comprises potentiating NPY's presence by inhibiting attractin or attractin isoforms. Oral administration of an attractin inhibitor may be preferable in most circumstances. By inhibiting the attractin enzyme activity, the half-life of the active form of NPY will be appreciably extended and maintained under physiological conditions. The extended presence of active NPY will enhance the NPY Y1 receptor activity.

This invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically (or prophylactically) effective amount of the inhibitor (and/or a sugar pill to accompany administration of an attractin inhibitor), and a pharmaceutically acceptable carrier or excipient, especially adapted for targeting the brain. Suitable carriers include but are not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The carrier and composition are preferably produced under good laboratory practices conditions and most preferably are sterile. The formulation is ideally selected to suit the mode of administration, in accordance with conventional practice.

Suitable pharmaceutically acceptable carriers include but are not limited to water, salt solutions (for example, NaCl), alcohols, gum arabic, vegetable oils, benzyl alcohols, polyethylene glycols, gelatin, carbohydrates such as lactose, amylose or starch, magnesium stearate, talc, viscous paraffin, perfume oil, fatty acid esters, hydroxymethylcellulose, polyvinyl pyrrolidone, etc. The pharmaceutical preparations can be sterilized and if desired mixed with auxiliary agents, for example, lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, flavoring and/or aromatic substances and the like which do not deleteriously react with the active compounds, but which improve stability, manufacturability and/or aesthetic appeal.

The compositions, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. In addition, the composition can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. In addition, the composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulations

can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, polyvinyl pyrrolidone, sodium saccharine, cellulose, magnesium carbonate etc.

Further, the compositions can be formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active compound. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water, saline or dextrose/water. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

Finally, compositions of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acid, etc., and those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the invention's composition which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, *in vitro* and/or *in vivo* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgement of the practitioner and each patient's circumstances.

It will be readily understood by the skilled artisan that numerous alterations may be made to the examples and instructions given herein including the generation of different attractin inhibitors and alternate therapeutic compositions without departing from either the spirit or scope of the present invention.

The present invention will now be illustrated with reference to the following example, focussing on the anxiolytic-like and stress-protective-like action of reduced attractin activity.

EXAMPLE

Example 1

NPY is a substrate for human attractin in vitro

Attractin from human plasma (Baxter GmbH Germany, Plasmazentrum Halle) was prepared from 100 ml plasma from healthy humans. Matrix-assisted laser desorption/ionisation mass spectrometry was carried out using the Hewlett-Packard G2025 LD-TOF System.

To obtain spectra of peptides (25 µM) by the treatment of purified DP IV and attractin in the presence or absence of isoleucyl-thiazolidine (10 µM), substrates were incubated at 37 °C with 40 mM tricine/HCl buffer pH 7.6 and enzyme solution in a 2:2:1 ratio. Samples of the reaction mixtures were removed at various time intervals and mixed with equal volumes of the matrix solution. By mixing assay sample and matrix, the low pH of the matrix solution stopped the enzymatic reaction. A small volume of this mixture was transferred to a probe tip and immediately evaporated in a Hewlett-Packard G2024A Sample Prep Accessory.

In further studies the proteolytic activity of attractin has been investigated. The K_m value for Gly-Pro-pNA was determined to be 0.14 mM, comparable to values found for DP IV, attractin and a serum DP IV-like activity. In addition, the cleavage of bioactive peptides such as NP Y, RANTES, GIP, and Glucagon by purified attractin has been analyzed. Similar to DP IV, attractin is capable of releasing the N-terminal dipeptide Tyr-Pro from NP Y (Fig. 1 B, left side). In presence of isoleucyl-thiazolidine, the cleavage was suppressed (Fig. 1 B, right side). Previously, differences within hydrolysis of the chemokine RANTES by DP IV and attractin have been described[4]. However, our results do not confirm these data. Similar to DP IV (Fig. 1A, left side), purified attractin is capable of releasing sequentially the first (Ser-Pro) as well as the second dipeptide (Tyr-Ser) from N-terminus of the synthetic $RANTES_{1-15}$ (Fig. 1A, right side).

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CLAIMS

1. Use of an inhibitor of attractin or of an attractin isoform for the production of a medicament for modulating behavioral and/or neurological responsiveness to stress including anxiety.

2. Use according to claim 1 for the production of a medicament for the reduction of degradation of the endogenous, CNS-localized neuropeptide Y (NPY) and other substrates sharing similar properties.

3. Use according to any one of the preceding claims for the production of a medicament for the treatment of psychosomatic, depressive and neuropsychiatric diseases.

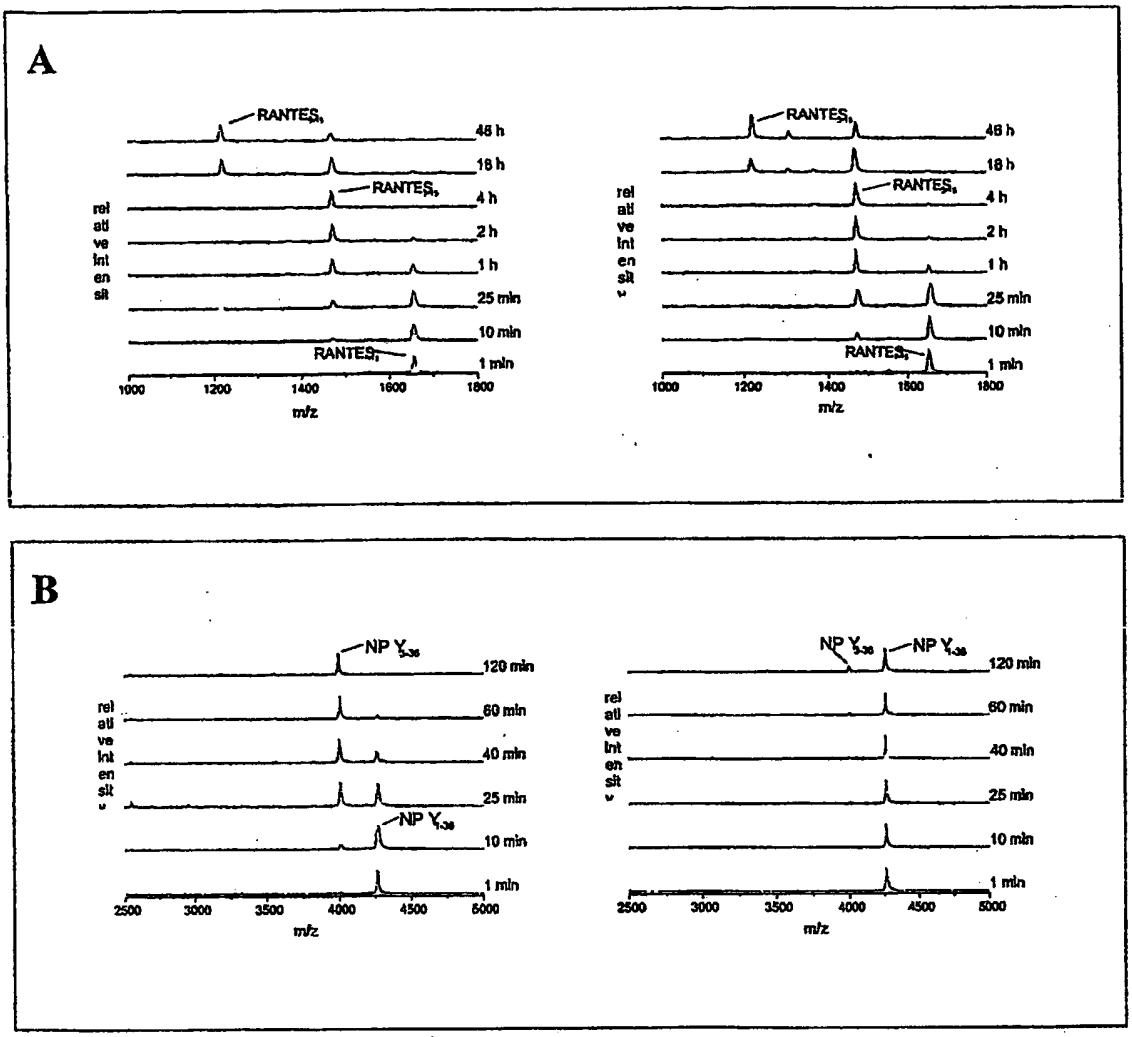
4. Use according to claim 3, characterized in that the psychosomatic, depressive and neuropsychiatric diseases are selected from the group consisting of anxiety disorders, depression, insomnia, chronic fatigue, schizophrenia, epilepsy, eating disorders, spasm and chronic pain.

5. Use according to any one of the preceding claims, characterized in that the inhibitors are used in combination with neuropeptide Y.

6. Use according to any one of the preceding claims, characterized in that the inhibitors are present in a physiologically compatible drug delivery vehicle.

7. Use according to any one of the preceding claims, characterized in that the inhibitors are formulated as prodrugs of the inhibitors.

8. Use according to any one of the preceding claims, characterized in that the inhibitors are used parenterally, enterally, orally, by inhalation or as a suppository.


A
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RANTES3-68
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RANTES1-68
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relative intensity
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Figur 1

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(72) Inventors; and
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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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— *with international search report*

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30 January 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/034242 A3

(54) Title: METHOD FOR THE TREATMENT OF NEUROLOGICAL AND NEUROPSYCHOLOGICAL DISORDERS

(57) Abstract: The present invention discloses a method for therapeutically treating an animal, including a human, for psychosomatic, depressive and neuropsychiatric diseases, such as anxiety, depression, insomnia, schizophrenia, epilepsy, spasm and chronic pain. Administration of a suitable attractin inhibitor causes the reduction of activity in the enzyme attraction or in isoforms thereof in the brain of mammals and leads as a causal consequence to a reduced degradation of the neuropeptide Y (NPY) and similar substrates. Such treatment will result in a reduction or delay in the decrease of the concentration of functionally active neuronal NPY (1-36). As a consequence of the resulting enhanced stability of the endogenous NPY(1-36), NPY activity is prolonged thereby resulting among other things in functionally active NPY Y1 receptor activity thereby facilitating antidepressive, anxiolytic, analgesic, antihypertension and other neurological effects.

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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/40 A61K31/425 A61K31/17 A61K31/00 A61P25/22
A61P25/18 A61P25/24

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, MEDLINE, BIOSIS, CHEM ABS Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	DATABASE CAS REGISTRY FILE 'Online! American Chemical Society (ACS); "Attractin/cn" Database accession no. RN : 54248-88-6 XP002210175 the whole document	1-8
T	"Topics" INTERNATIONAL CONFERENCE ON DIPEPTIDYL AMINOPEPTIDASE, 'Online! XP002210174 Berlin, Germany Retrieved from the Internet: <URL:http://www.dppiv.com/seiten/topics.html> 'retrieved on 2002-08-16! paragraphs '0001!,'0002! -/--	1-8

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 September 2002	18/10/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Bazzanini, R

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INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 00 53171 A (OGNIBENE AGOSTINO ;ROTELLA CARLO MARIA (IT); MANNUCCI EDOARDO (IT)) 14 September 2000 (2000-09-14) page 1, line 5-21	1-8
Y	WETTSTEIN J G ET AL: "Central nervous system pharmacology of neuropeptide Y." PHARMACOLOGY & THERAPEUTICS. ENGLAND MAR 1995, vol. 65, no. 3, March 1995 (1995-03), pages 397-414, XP001097134 ISSN: 0163-7258 abstract page 399, paragraph 5 page 400, paragraph 7 page 402, paragraphs 2,3 page 403, paragraph 3 page 404, paragraph 2 page 406, paragraphs 5,7 page 407, paragraphs 1,3	1-8
Y	MUNGLANI R ET AL: "THE THERAPEUTIC POTENTIAL OF NEUROPEPTIDE Y ANALGESIC, ANXIOLYTIC AND ANTIHYPERTENSIVE" DRUGS, ADIS INTERNATIONAL LTD, AT, vol. 52, no. 3, September 1996 (1996-09), pages 371-389, XP002943797 ISSN: 0012-6667 abstract page 375, left-hand column, paragraph 1 page 375, right-hand column, paragraph 2 page 379, right-hand column, paragraph 3 page 380, last line, paragraphs 3-5 page 382, left-hand column, paragraph 5 -right-hand column, paragraph 3	1-8
Y	BADIA-ELDER N E ET AL: "Effects of neuropeptide Y (NPY) on ethanol intake and anxiety in high and low alcohol drinking (HAD1/LAD1) rats." ALCOHOLISM CLINICAL AND EXPERIMENTAL RESEARCH, vol. 24, no. 5 Supplement, May 2000 (2000-05), page 82A XP008006544 Scientific Meeting of the Research Society on Alcoholism;Santa Barbara, California, USA; June 24-29, 2000 ISSN: 0145-6008 the whole document	1-8

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INTERNATIONAL SEARCH REPORT

Int nal Application No
PCT/EP 01/12478

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DE 299 09 210 U (PROBIODRUG GES FUER ARZNEIMITT) 9 September 1999 (1999-09-09) page 3, paragraph 3 page 5, line 4 table 1 examples 1,2 page 20, paragraph 4 page 21, paragraph 3 claims 1-9	1-8

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-8 relate to a compound defined by reference to a desirable characteristic or property, namely "inhibitor of attractin or of an attractin isoform" and "prodrugs".

The claims cover all compounds having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT for only a very limited number of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful complete search over the whole of the claimed scope is impossible.

Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compound by reference to its pharmacological profile, rendering the scope of protection of said claims obscure (Article 6 PCT). It is pointed out that a compound cannot be sufficiently characterized by its pharmacological profile or its mode of action or metabolism. The use of such a functional definition is vague and unclear and leaves the reader in doubt as to the meaning of the technical feature (i.e. compounds) to which it refers. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

Moreover claims 1-3,5-8 relate to the treatment of a disease which actually is not well defined.
The use of the definitions " behavioral and/or neurological responsiveness to stress" (claim 1), "reduction of degradation of the endogenous, CNS-localized neuropeptide (NPY) and other substrates sharing similar properties" (claim 2) and "psychosomatic/neuropsychiatric diseases" (claim 3) is considered to lead to a lack of clarity within the meaning of Article 6 PCT. It is impossible to determine the diseases for which protection might legitimately be sought. The lack of clarity is such as to render a meaningful complete search impossible.

Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the compounds mentioned in the description at page 7, paragraph 7 (i.e. L-threo-isoleucyl thiazolidine, L-allo-isoleucyl thiazolidine, L-threo-isoleucyl pyrrolidine, L-allo-isoleucyl-pyrrolidine), and those parts relating to the diseases mentioned in the description on page 1, lines 5-10 also reported in claims 1,3,4 (i.e. anxiety, depression, insomnia, chronic fatigue, schizophrenia, epilepsy, eating disorders, spasm and chronic pain, hypertension, fever, anorexia, drug withdrawal, alcoholism, dementia, congnitive dysfunctions), with due regard to the general idea underlying the invention.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

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INTERNATIONAL SEARCH REPORT

national application No.
PCT/EP 01/12478

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

formation on patent family members

In onal Application No
PCT/EP 01/12478

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
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			EP	1215207 A2	19-06-2002
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